

## Article

# Directed Microtubule Growth, +TIPs, and Kinesin-2 Are Required for Uniform Microtubule Polarity in Dendrites

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## Summary

**Background:** In many differentiated cells, microtubules are organized into polarized noncentrosomal arrays, yet few mechanisms that control these arrays have been identified. For example, mechanisms that maintain microtubule polarity in the face of constant remodeling by dynamic instability are not known. *Drosophila* neurons contain uniform-polarity minus-end-out microtubules in dendrites, which are often highly branched. Because undirected microtubule growth through dendrite branch points jeopardizes uniform microtubule polarity, we have used this system to understand how cells can maintain dynamic arrays of polarized microtubules.

**Results:** We find that growing microtubules navigate dendrite branch points by turning the same way, toward the cell body, 98% of the time and that growing microtubules track along stable microtubules toward their plus ends. Using RNAi and genetic approaches, we show that kinesin-2, and the +TIPS EB1 and APC, are required for uniform dendrite microtubule polarity. Moreover, the protein-protein interactions and localization of Apc2-GFP and Apc-RFP to branch points suggests that these proteins work together at dendrite branches. The functional importance of this polarity mechanism is demonstrated by the failure of neurons with reduced kinesin-2 to regenerate an axon from a dendrite.

**Conclusions:** We conclude that microtubule growth is directed at dendrite branch points and that kinesin-2, APC, and EB1 are likely to play a role in this process. We propose that kinesin-2 is recruited to growing microtubules by +TIPS and that the motor protein steers growing microtubules at branch points. This represents a newly discovered mechanism for maintaining polarized arrays of microtubules.

## Introduction

Most cells in multicellular organisms contain polarized noncentrosomal microtubule arrays. In interphase mammalian cultured cells, microtubules are nucleated at the centrosomal microtubule-organizing center (MTOC), and plus ends grow toward the cell periphery. However, in many differentiated cells, minus ends are not focused at a centrosomal MTOC. In epithelial cells, a major population of microtubules has minus ends focused at the apical side and plus ends at the basal side. In muscle cells, minus ends spread out around the nuclear envelope, and neurons have perhaps the simplest and most strikingly polarized noncentrosomal microtubule

arrays [1, 2]. The mechanisms that organize these noncentrosomal microtubule arrays are poorly understood.

Neurons have two types of processes that extend from the cell body: axons and dendrites. Dendrites primarily receive signals from other neurons or the environment, and axons send signals to other neurons or output cells. One basic difference between axons and dendrites is the arrangement of microtubules. In axons microtubules are arranged into an overlapping array of uniform-polarity plus-end-out microtubules [3–7]. In dendrites of cultured mammalian neurons, microtubules have mixed orientation near the cell body [7, 8]. In dendrites of *Drosophila* neurons, 90%–95% of microtubules have minus ends distal to the cell body [9–12]. Because the dendritic array in *Drosophila* is very simple, and extremely different from a centrosomal array, we used it as a model system to identify mechanisms that organize polarized noncentrosomal microtubules.

It is not known how uniform dendrite microtubule polarity is established or maintained. Models for generating the plus-end-out axonal microtubule array focus on the sliding of microtubules by motor proteins. In mammalian neurons, microtubules are thought to be nucleated in the cell body at the centrosome [13], then released from the centrosome and transported down the axon in the correct orientation by motors, including dynein [14, 15]. Models that account for mixed orientation microtubules in dendrites of cultured neurons have also been proposed. In this case, the kinesin MKLP1 has been proposed to transport minus-end-out microtubules into dendrites along plus-end-out microtubules [16]. In the current study, we identify a new mechanism that is required for uniform microtubule polarity in dendrites. Because it uses conserved, generally expressed proteins, it could play a role in maintaining microtubule polarity in many other cell types.

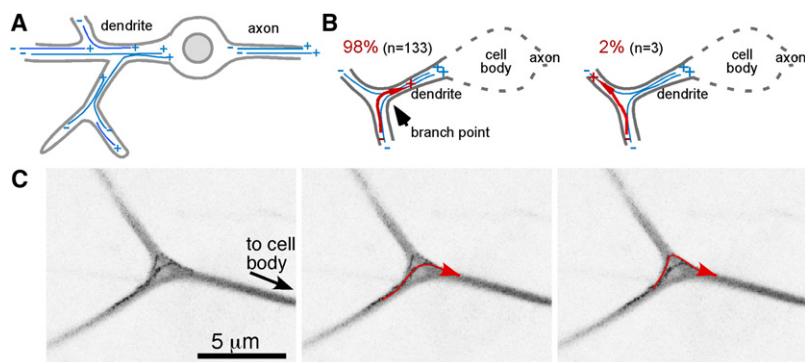
## Results

### Directed Growth of Microtubules at Dendrite Branch Points

In branched dendrites with minus-end-out microtubules (Figure 1A), undirected microtubule growth threatens uniform microtubule polarity. If microtubule plus ends extend from a distal dendrite through a branch point into the other distal dendrite, then a plus-end-out microtubule will be present in the recipient dendrite (Figure 1B, right diagram). We therefore assayed microtubule growth through dendrite branch points by visualizing growing plus ends with neuronally expressed EB1-GFP. EB protein dynamics have been successfully used for imaging growing microtubules in both *Drosophila* and mammalian neurons [8, 9, 17]. In all cases the tagged EB proteins bind primarily to the growing plus ends of microtubules.

In *Drosophila*, EB1-GFP dynamics can be visualized in the branched dendritic arborization (da) neurons of the larva. In these neurons the dendritic tree is sandwiched between the cuticle and epithelial cells and so is essentially two dimensional, which is ideal for microscopic analysis [18, 19]. All of our live imaging experiments were performed in the dorsal cluster of da neurons.

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**Figure 1. Microtubules Grow Toward the Cell Body at Dendrite Branch Points**

(A) The layout of stable microtubules in da neurons is shown.

(B) Growing microtubules (red) turn either into a distal dendrite (left) or toward the cell body (right). Quantitation above the diagrams is based on analysis of EB1-GFP dynamics as in (C).

(C) EB1-GFP was expressed in da neurons with elav-Gal4, and movies of growing microtubules in larval da neurons were acquired with a confocal microscope. For this figure, 16 frames were projected into a single image with a maximum-projection method, and the image lookup table was inverted. Paths of two microtubules can be seen, and are highlighted in red. See also [Movie S1](#).

When microtubules that extended through branch points were assayed, 98% grew toward the cell body (Figure 1B). The path of a growing microtubule could curve gently through the branch point, or a microtubule plus end could make a sharp turn (Figure 1C; see also [Movie S1](#)). Because the behavior of growing microtubules at branch points is not random, microtubule growth must be directed by an active mechanism.

#### Plus Ends of Growing Microtubules Track Stable Microtubules

To understand how microtubule growth could be directed through dendrite branch points, we observed sequential microtubules growing through the same branch. Microtubules frequently followed exactly the same route through a branch point (Figure S1 and Movie 2). We hypothesized that growing microtubules might be recognizing some structure that passed through the branch and served as a track.

For a track to direct microtubules reproducibly toward the cell body, it would need to have an intrinsic polarity. The most obvious long, polarized structures in cells are microtubules. To determine whether microtubule growth could be guided by an existing microtubule, we visualized stable microtubules by using a protein-trap transgenic line that contains the GFP coding sequence inserted into the endogenous *Drosophila* tau gene [9] together with growing microtubules labeled by EB1-RFP. EB1-RFP labels growing microtubule plus ends but also localizes to other larger structures that move much more rapidly through dendrites, or that do not move at all, and that are not seen with EB1-GFP; we think these other structures represent nonspecific labeling.

When we performed two-color live imaging of animals expressing tau-GFP and EB1-RFP, we observed microtubule plus ends coinciding with stable microtubules (Figure 2; see also [Movie S3](#) and [Movie S4](#)). The tau-GFP signal was low and susceptible to photobleaching, but because its pattern was stable over time, we used a single frame to ascertain the layout of stable microtubules. Particularly informative were large branch points with distinctive patterns of stable microtubules running through them. In these cases, we could clearly see that the EB1-RFP followed along the stable microtubule track.

#### The Kinesin-2 Motor Is Required for Uniform Microtubule Polarity in Dendrites

Because growing microtubule plus ends in dendrites can use stable microtubules as tracks and travel toward their plus end (Figure 1B), kinesins that walk toward microtubule plus ends are good candidates for mediating directed growth. For

a kinesin to play this role, it would need to interact with the growing plus end. Khc73, a kinesin-3 family member, has been shown to localize to microtubule plus ends in *Drosophila* neuroblasts [20]. In mammalian cells, a kinesin-2 (KIF3) subunit, Kap3, can interact with the microtubule-plus-end-binding protein (+TIP) adenomatous polyposis coli (APC) [21]. We therefore tested kinesin-2 and Khc73 for a role in directed microtubule growth.

Kinesin-2 is a heterotrimeric kinesin with two different motor subunits, named Klp64D and Klp68D in *Drosophila*, and an accessory subunit, Kap3. Using RNAi, we reduced levels of each of the kinesin-2 subunits or Khc73 in da neurons expressing EB1-GFP. To perform cell-type-specific RNAi, we expressed UAS-hairpin RNAs, as well as EB1-GFP, under control of a neuronal Gal4 driver. We also included a UAS-Dicer2 transgene because this can increase RNAi effectiveness in *Drosophila* neurons [22].

If directed growth of microtubules at branch points requires either of these kinesins, then microtubule polarity in the dendrite should change when their levels are reduced. We used movies of EB1-GFP dynamics in dorsal cluster neurons to assay microtubule orientation by scoring the direction of movement of EB1-GFP comets. In control (Rtnl2) RNAi experiments, 1.2% of comets moved away from the cell body, representing plus-end-out microtubules, and the rest moved toward the cell body at the plus ends of minus-end-out microtubules. We used Rtnl2 RNAi as a control because we never observe any phenotypes when compare cells expressing this RNAi to cells expressing no RNAi hairpin. In dendrites in which any of the three kinesin-2 subunits were targeted by RNAi, the number of plus-end-out microtubules was increased to 20%–28% (Figure 3; see also [Movie S5](#) and [Movie S6](#)). To analyze the specificity of the phenotype, we analyzed microtubule orientation in proximal axons of neurons expressing control or Kap3 RNAi hairpins. In both conditions microtubules were almost exclusively plus-end out (Figure 3C).

To confirm the RNAi results, we also assayed microtubule orientation in *Klp64D* mutants. We used a combination of hypomorphic *Klp64D* alleles, *Klp64D<sup>k1</sup>/Klp64D<sup>k5</sup>*, and assayed EB1-GFP dynamics in this background. Previous phenotypic characterization of *Klp64D* alleles determined that animals with this mutant combination had defects only in ciliated sensory neurons [23]. As in the RNAi experiments, we found that the proportion of plus-end-out microtubules was increased to 29% in *Klp64D* mutant animals (Figure 3B). In addition, we used a different assay to test whether kinesin-2 might have a role in microtubule organization in dendrites of central neurons (see [Figure S2](#)). All experiments were

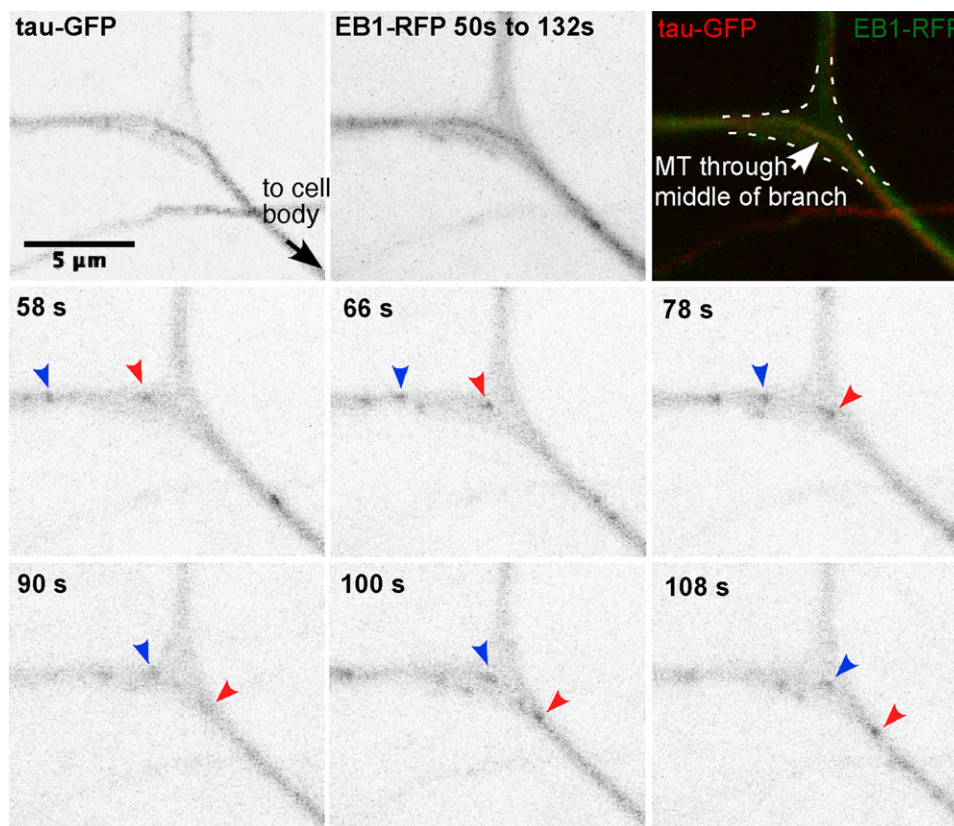


Figure 2. Growing Microtubule Plus Ends Track Along Stable Microtubules through Dendrite Branch Points

Movies of da neurons in larvae expressing EB1-RFP (arrows) and Tau-GFP were acquired with a confocal microscope. A maximum projection of frames from 50 s to 132 s is shown (top middle panel). Superposition of the EB1-RFP projection and Tau-GFP image shows that the microtubule plus end in the branch follows the path of the middle microtubule track labeled with Tau-GFP (top right panel). See also [Figure S1](#), [Movie S2](#), [Movie S3](#), and [Movie S4](#).

consistent with a specific role for kinesin-2 in controlling dendrite microtubule polarity.

#### Dendrite Branch Angle Correlates with the Severity of the Kinesin-2 Phenotype

Having shown that kinesin-2 is required for uniform microtubule polarity in dendrites, we wished to determine whether the geometry of dendrite branch points might also be important for polarity. Four classes of da neurons are present in the larval body wall, and each has its own distinctive branching pattern [19]. One of these cells, the class I neuron ddaE, has a dendrite with branch angles closer to right angles than other dendrites. We measured the turns growing microtubules would need to make in this ddaE comb-like dendrite compared to dendrites of class II-IV neurons. We found that in class II-IV neurons the turn angle toward the cell body was much smaller than that away from the cell body, meaning that the geometry of the branch favored growth toward the cell body. In the comb dendrite these two angles were much more similar ([Figure 4A](#)). If directed growth at dendrite branch points contributes to microtubule polarity in dendrites, then we should observe stronger polarity defects in the comb dendrite when kinesin-2 levels are reduced.

We performed RNAi to reduce kinesin-2 subunits and focused on the comb dendrite of the class I neuron ddaE. More plus-end-out microtubules were present in the comb dendrite ([Figure 4B](#), dark bars) than in other dendrites ([Figure 4B](#), light bars) when each of the kinesin-2 subunits was

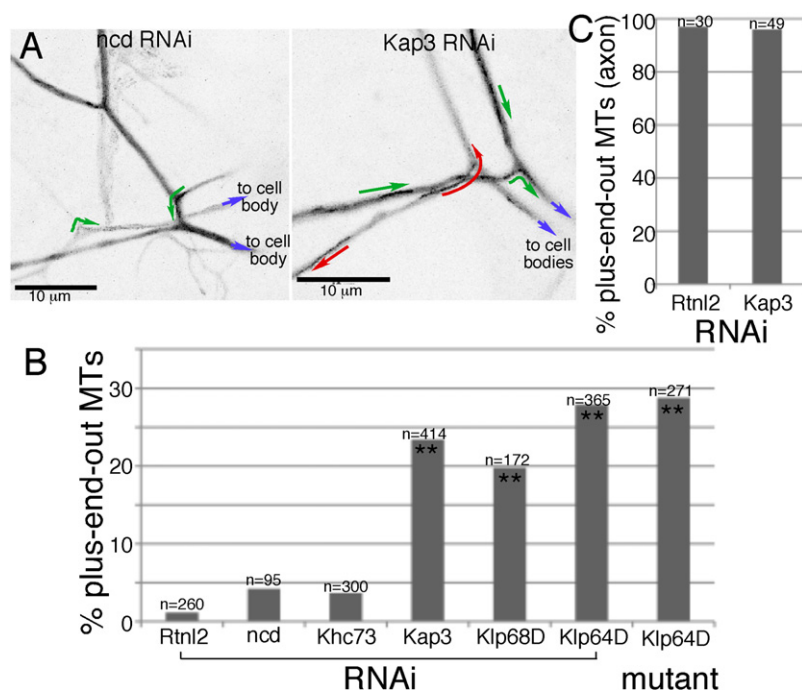
targeted. In fact, microtubule orientation was completely randomized in the comb dendrite when Klp64D was targeted ([Figure 4B](#); see also [Movie S7](#) and [Movie S8](#)). These results offer an explanation for why only 20%–28% of microtubules are plus-end out in dendrites of class II-IV neurons with reduced levels of kinesin-2: the branch angles in these dendrites most likely also help to channel growing microtubules toward the cell body.

Despite this large change in microtubule polarity, overall dendrite shape and branch number were not changed in ddaE ([Figures S3A](#) and [S3B](#)). We also analyzed the speed of EB1-GFP comet movement in control and Kap3 RNAi dendrites and found this to be the same in both conditions ([Figure S3C](#)). To make sure that movements in both directions were consistent with microtubule plus-end growth, we separated speed data in Kap3 RNAi dendrites into groups based on direction of comet movement. Both groups had very similar rates of movement ([Figure S3C](#), right) and were consistent with rates of microtubule plus-end growth stimulated by msp/XMAP215 [24], which participates in microtubule growth in *Drosophila* neurons [25]. Thus, kinesin-2 specifically affects microtubule polarity and not other parameters of microtubule growth.

#### +TIP Proteins Are Required for Maintenance of Uniform Microtubule Polarity in Dendrites

To identify proteins that could mediate an interaction between kinesin-2 and growing microtubule plus ends, we reduced





**Figure 3. Reduction of Kinesin-2 Subunits Increases the Proportion of Plus-End-Out Microtubules in Dendrites**

The *elav-Gal4* pan-neuronal driver and UAS-hairpin lines from VDRC were used for cell-type specific RNAi in neurons; neurons also expressed *dicer2* and EB1-GFP. (A) Maximum-value projections of EB1-GFP in da neuron dendrites. Green or red arrows indicate the direction of individual EB1-GFP comets. Blue arrows indicate the position of the cell body. (B) The direction of EB1-GFP comet movement was quantitated in larvae expressing different RNAi hairpins and in *Klp64D* mutant animals (genotype *elav-Gal4/UAS-EB1-GFP; Klp64D<sup>K1</sup>/Klp64D<sup>K5</sup>*). n's indicate the number of EB1-GFP comets assayed in each genotype. Double stars indicate statistically significant differences from control *Rtnl2*, at  $p < 0.0001$  via Fisher's exact test. (C) Microtubule orientation in control and *Kap3* RNAi axons was assayed as above with EB1-GFP. Quantitation was performed as in dendrites. See also [Figure S2](#), [Movie S5](#), and [Movie S6](#).

levels of +TIP proteins in da neurons and assayed microtubule orientation. The +TIP EB1 is proposed to be a core plus-end binding protein that mediates interaction of other proteins with growing plus ends [26–29] and is likely to be involved in most microtubule plus-end behaviors. To assay microtubule polarity without relying on EB1, we used a previously developed assay that uses Rab4-RFP to reveal patterns of membrane transport at dendrite branch points [9]. Microtubules that extend from one distal dendrite into another at a branch point (bridging microtubules) must have a plus end out in one dendrite and a minus end out in the other, meaning that one of the dendrites has mixed polarity (Figure 5A). In control RNAi experiments in da neurons, 6.9% of Rab4-labeled particles moved from one distal dendrite into the other, most likely on bridging microtubules. The remainder moved from dendrite to cell body or vice versa. When EB1 was targeted by RNAi, the number of Rab4 particles moving between dendrites basically doubled, to 15.1% (Figure 5A). These results are consistent with an increased number of dendrites with mixed polarity in neurons depleted of EB1.

To confirm a role for EB1 in dendrite microtubule orientation, we overexpressed EB1-GFP in da neurons and used direction of comet movement to assay microtubule orientation. Many +TIPs, including CLIP proteins and APC, are recruited to growing microtubules by binding to the C terminus of EB1 [28]. Addition of GFP to the C terminus of EB proteins can block these interactions [30]. In most experiments, we expressed EB1-GFP at low levels in all neurons by using one copy of *elav-Gal4*. To drive higher levels of expression, we used one or two copies of the stronger 109(2)80 *Gal4* driver, which is expressed in a subset of da neurons. At very high expression levels, some EB1-GFP accumulated along the length of the microtubule as well as at the tip (Movie S9), but we were still able to assay the direction of comet movement. At the normal low expression level, 4.8% of EB1-GFP comets moved away from the cell body, representing plus-end-out microtubules. At moderate expression levels, with one copy of 109(2)80, this

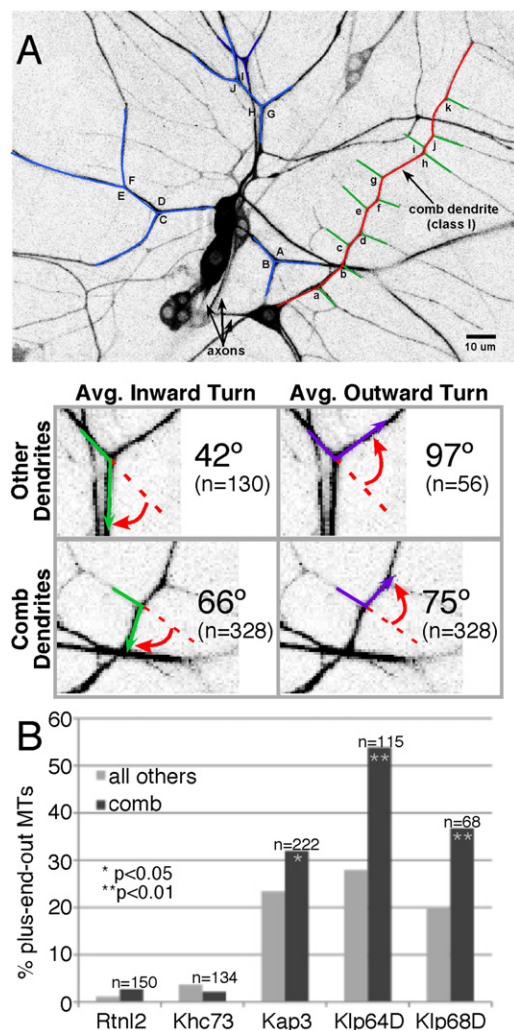
was increased to 10.8%, and at very high levels it was increased further to 18.1% (Figure 5B). As an additional method to determine whether EB1 contributes to dendrite microtubule polarity, we expressed a C-terminal fragment of EB1 (EB1-CT) in da neurons; a similar region

of mammalian EB1 has been shown to have dominant-negative (DN) activity [31, 32]. In the comb dendrite of the *ddaE* cell, EB1-CT expression increased the number of plus-end-out microtubules (Figure 5D). These results are consistent with a role for EB1 in maintaining uniform dendritic microtubule polarity.

In mammals the major neuronal EB family member is EB3. In *Drosophila*, EB1 is the protein most similar to mammalian EB1 and EB3, so *Drosophila* EB1 most likely fulfills the functions of both EB1 and EB3. There are, however, additional genes that have sequence similarity to EB1 in the *Drosophila* genome [33]. We targeted the most similar of these EB1-like genes, CG32371, by RNAi, and the results were similar to control results (Figure 5B).

Because *Kap3* and EB1 have both been reported to bind to APC in mammals, we also wished to test a role for APC in organizing dendritic microtubules. In *Drosophila* there are two APC proteins, *Apc* and *Apc2*. They have overlapping roles in the nervous system [34], so we generated a *Apc2<sup>N175K</sup>, Apc<sup>Q8</sup>* homozygous double-mutant larva that expressed EB1-GFP in neurons. In dendrites of class II-IV da neurons, the number of plus-end-out microtubules was significantly elevated in mutants (Figure 5C). The phenotype was not as strong as when kinesin-2 was targeted, perhaps because of the stability of maternally loaded APC proteins. When we examined the phenotype in the comb-shaped dendrite of the class I *ddaE* cell, the percentage of plus-end-out microtubules increased in double mutants dramatically, from 9% in the heterozygote to 32% in the homozygote (Figure 5C). This increase in phenotype strength in the comb dendrite was similar to that seen with kinesin-2 RNAi.

We used neuron-specific RNAi to determine whether APC proteins have a cell-autonomous role in controlling dendrite microtubule polarity. Targeting *Apc*, *Apc2*, or both by RNAi resulted in an increase in plus-end-out microtubules in the comb dendrite (Figure 5D). We conclude that EB1, *Apc*, and *Apc2* are all required to maintain uniform microtubule polarity in dendrites.

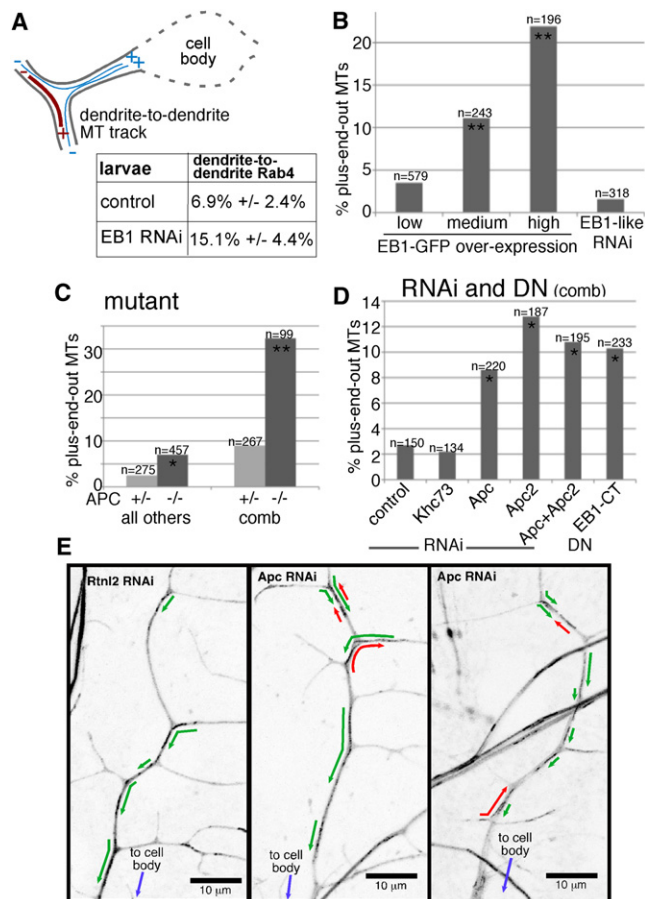


**Figure 4. Dendrite Branching Angle Determines Severity of Kinesin-2 Phenotype**

(A) Highlighted examples of class I (red & green) and non-class I (blue) neurons are shown. Red indicates the main trunk of the class I neuron. The angle that a growing microtubule (green and purple arrows) would be required to turn at a dendrite branch point (red arrow) was measured in the comb dendrite of *ddaE*, the class I *da* neuron, and all other *da* neurons. (B) RNAi and EB1-GFP analysis was performed as in Figure 3, except that EB1-GFP direction was quantitated in the main trunk of the *ddaE* dendrite only (dark bars in graph). For comparison, the light bars are data from other dendrites shown in Figure 3. *p* values (Fisher's exact test) indicate the level of significance for the difference between the comb and other data for a particular RNAi knock down. In all cases, phenotypes were significantly stronger in comb dendrites. See also Figure S3, Movie S7, and Movie S8.

#### An Interaction Network Links Kinesin-2 to +TIPs

Having shown that kinesin-2, EB1, Apc, and Apc2 are all required for dendrite microtubule polarity, we wished to determine whether any of them might physically interact. Mammalian Kap3 was previously reported to interact with the N-terminal region of APC that contains Armadillo repeats (ARM domain) [21]. We found that the ARM domain of *Drosophila* Apc, but not Apc2, could interact with *Drosophila* Kap3 in a two-hybrid assay (Figure 6B). We also found that the ARM domains of Apc and Apc2 could interact with one another, and the C terminus of Apc interacted with the C terminus of EB1. In addition, Apc and Klp68D contain the newly



**Figure 5. EB1 and APC Are Required for Maintenance of Uniform Microtubule Polarity in Dendrites**

(A) Rab4-RFP-labeled membranes that moved smoothly through a branch point were classified into two categories: those moving from dendrite to dendrite and those moving between the cell body and dendrite (table). Error margins indicate standard deviation; the difference between the EB1 RNAi and the wild-type was statistically significant according to an unpaired *t* test ( $p < 0.05$ ).

(B) EB1-GFP was expressed in neurons at low levels with *elav-Gal4*, at medium levels with one copy of 109(2)80, and at high levels with two copies of 109(2)80. Direction of EB1-GFP movement in dendrites was quantitated. The differences between medium and high EB1-GFP expression and low expression were statistically significant (\*\*) according to a Fisher's exact test ( $p < 0.0001$ ). See also Movie S9. Also included are results from RNAi targeting the EB1-like gene CG32371 and performed as in Figure 3.

(C) EB1-GFP was expressed with *elav-Gal4* in animals homozygous and heterozygous for the double-mutant combination *Apc2<sup>N175K</sup>*, *Apc<sup>Q8</sup>* (APC in table). The difference between the double mutant and heterozygote was statistically significant in a Fisher's exact test in the comb dendrite ( $p < 0.0001$ , \*\*) and other dendrites ( $p < 0.05$ , \*).

(D) EB1-GFP, Dicer2, and either hairpin RNA or EB1-CT expression was driven with *elav-Gal4*. The direction of comet movement was scored in the main trunk of the *ddaE* comb dendrite as in Figure 4. Compared to control (*rtnl2* RNAi) neurons, the indicated conditions were significantly different ( $p < 0.05$ , \*).

(E) Maximum-value projections of EB1-GFP in *da* neuron dendrites in larvae in which Rtnl2 or Apc was targeted by RNAi are shown. Green or red arrows indicate the direction of EB1-GFP comets.

identified SxIP motif, which can mediate interactions of proteins with EB1 [29]. The interactions identified by two-hybrid analysis place Apc at the center of a network that links kinesin-2 to EB1 (Figure 6E).

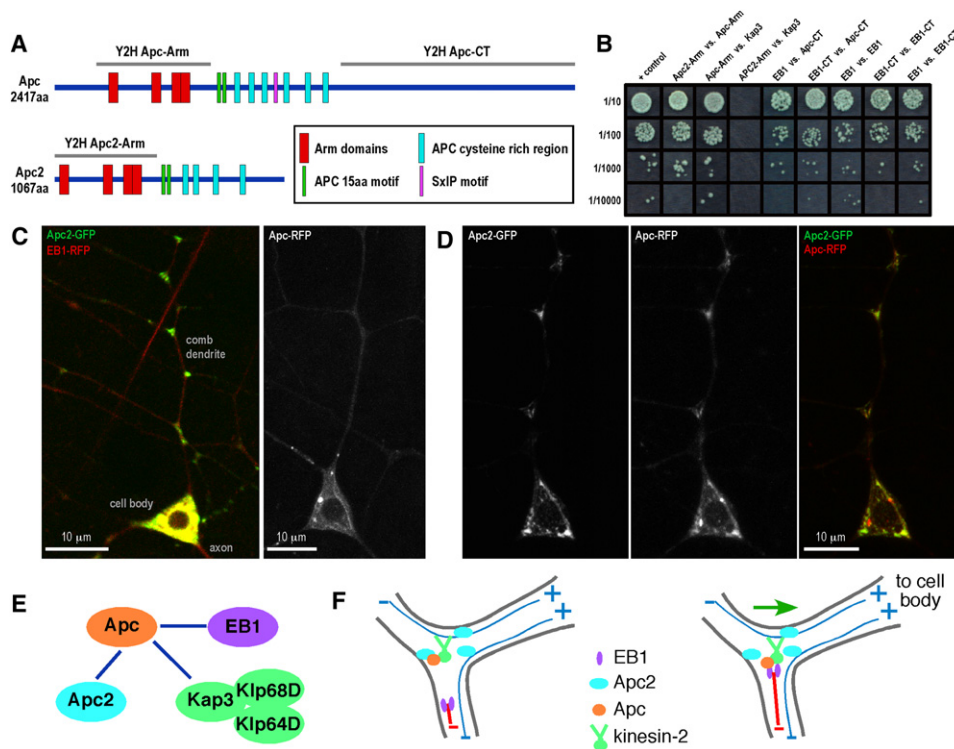


Figure 6. Interactions between Kinesin-2 and +TIPs and Localization of Apc2-GFP to Dendrite Branch Points

(A) Schematics of domains in the *Drosophila* Apc and Apc2 proteins are shown. The Arm domain region of both proteins and the C-terminal domain of Apc were used for two-hybrid analysis, as was full-length *Drosophila* Kap3, full-length EB1, and the C-terminal domain of EB1.  
(B) Results of the two-hybrid analysis.  
(C) Apc2-GFP and EB1-RFP (for background) were expressed in class I da neurons with 221-Gal4 in the cell in the left panel. Apc-RFP was expressed alone in the cell in the right panel. The cell body and comb dendrite of ddaE are shown.  
(D) Apc-RFP and Apc2-GFP were expressed together in ddaE.  
(E) An interaction summary based on two-hybrid analysis is shown.  
(F) Model: See discussion. Growing microtubule is shown in red; stable microtubules are shown in blue.

### Apc2 Localizes to Dendrite Branch Points and Can Recruit Apc

We previously found GFP-tagged Apc2 localized to dendrites in *Drosophila* neurons [10]. In da neurons Apc2-GFP was highly concentrated at branch points (Figure 6C). It was also present in the cell body and sometimes in proximal axons, as previously reported [10, 25]. We conclude that Apc2 contains a signal for localization to dendrite branch points.

To test whether Apc2 could recruit other proteins to dendrite branch points, we expressed Apc2-GFP together with Apc-RFP. Apc-RFP when expressed on its own localized diffusely throughout the entire neuron, and there was some concentration on structures that look like microtubules (Figure 6C). When Apc-RFP was expressed in neurons with Apc2-GFP, it colocalized to large spots in the cell body and was enriched at dendrite branch points (Figure 6D). We conclude that Apc2 contains a signal that targets it to dendrite branch points to which it can recruit Apc.

### Kinesin-2 Is Required for Regeneration of an Axon from a Dendrite

As well as determining how kinesin-2 might contribute to dendrite microtubule polarity, we wanted to test the physiological importance of directed microtubule growth. General neuronal defects were not observed when kinesin-2 levels were reduced, so we wanted to test whether phenotypes

would arise under conditions that challenge neuronal polarity. Regeneration of an axon from a dendrite is a type of regeneration that requires polarity reversal and is conserved from *Drosophila* to mammals [35, 36].

We have previously shown that axon removal induces mixing of microtubule polarity throughout the dendritic tree [35]. If a single dendrite takes on the axonal plus-end-out microtubule polarity, and the remainder return to minus-end-out polarity, then growth of the process with axonal polarity is initiated [25]. We expressed an RNAi hairpin to target Klp64D and performed axon removal in vivo with a pulsed UV laser as previously described [25]. In control animals that do not express RNAi hairpins, about 2/3 of ddaE neurons reverse polarity of a single dendrite and initiate tip growth (Figure 7 and [25]). None of the six Klp64D RNAi neurons tested initiated normal tip growth (Figure 7). Four did not grow at all, and two had short new regions that were first seen at 96 hr. We quantitated microtubule orientation in the dendrite closest to the site of the axon because it is most often this dendrite that is converted to an axon [25]. In control neurons, this dendrite acquired axonal plus-end-out polarity about 50% of the time by 48 hr after injury. In Klp64D RNAi neurons this dendrite remained mixed (Figure 7). Thus, in Klp64D RNAi neurons, the failure to establish uniform polarity in a dendrite correlated with failure to initiate axon regeneration from a dendrite.



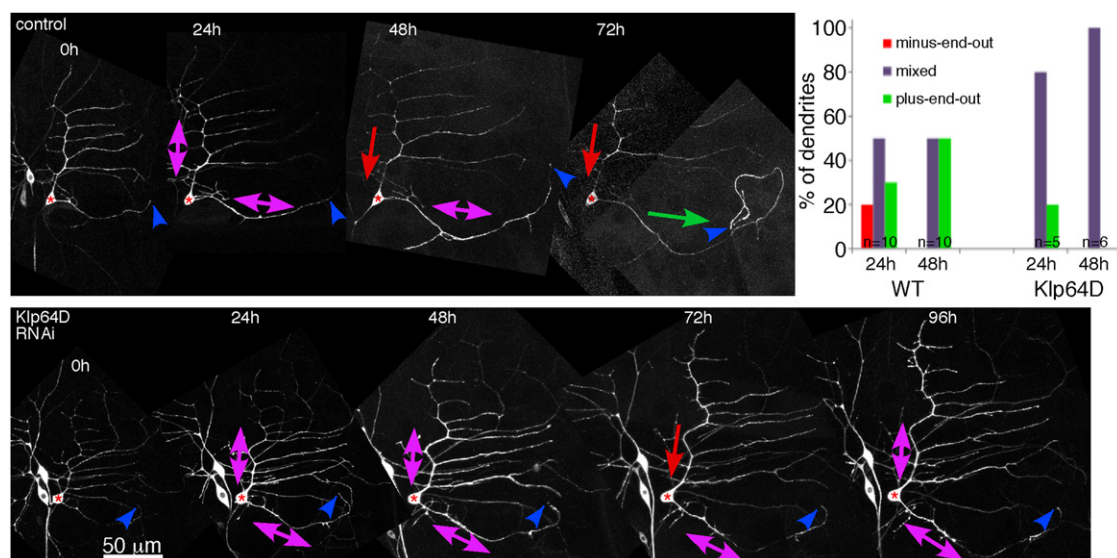


Figure 7. Kinesin-2 Is Required for Initiating Growth from the Tip of a Dendrite after Axon Removal

Axons of *ddaE* neurons (red stars) expressing EB1-GFP were severed close to the cell body at time 0 with a pulsed UV laser. We then imaged neurons briefly every 24 hr to determine whether they initiated growth from the tip of a dendrite and to evaluate dendrite microtubule polarity (arrows). Normal cells (top panel) initiated growth from the tip (blue arrowheads) of the dendrite nearest the site of the axon between 48 and 72 hr more than 50% of the time. Neurons expressing Klp64D hairpins (lower panel) had never initiated growth from this dendrite tip (blue arrowheads) by 48 or 72 hr. Two out of six had very short regions of growth by 96 hr. The scale is the same in both panels. The graph shows quantification of microtubule orientation in the dendrite closest to the site of the axon.

## Discussion

### A Model for Directed Microtubule Growth in Dendrites

Using *Drosophila* dendrites as a model system, we demonstrate that growing microtubule plus ends almost always turn toward the cell body at branch points and that they track stable microtubules through branches. Kinesin-2, EB1, and APC are all required for maintaining microtubule polarity and are linked in an interaction network.

On the basis of these results, we propose a model for directed growth of microtubules in dendrites. Apc2 most likely contains a branch localization signal because Apc2-GFP localizes well to dendrite branches even when overexpressed (Figure 6C). Localization of Apc2 to dendrite branch points can recruit Apc to the branch (Figure 6D). We show that Apc can interact with the Kap3 subunit of kinesin-2 and so could increase concentration of the motor near the branch. EB1-GFP does not concentrate at branches, so we propose that a growing microtubule plus end coated with EB1 is transiently linked, through the interaction between Apc and the EB1 tail, to kinesin-2 as it passes through the branch. SxIP motifs in Apc and Klp68D could also contribute to this interaction. Because both Kap3 and the SxIP motif in Klp68D are in the kinesin-2 tail, the motor domain would be free to walk along a nearby stable microtubule toward the plus end and cell body (Figure 6F).

Even a very brief application of force pulling the growing microtubule toward the cell body should be sufficient to steer growth toward the cell body. Once the tip of the microtubule turns, growth should be constrained by the dendrite walls. The association of the growing plus end with a stable microtubule would probably only need to be maintained over a distance of a micron. This model is consistent with the observations that kinesin-2 has shorter run lengths than kinesin-1

[37] and that individual EB1 interactions with the microtubule plus end persist for less than a second [38].

Observations of plus-end behavior *in vivo* also favor a model in which only transient interactions of the motor and microtubule plus end are involved; we frequently see plus ends turning sharply, as in Figure 1B. Stable microtubules do not accommodate such sharp turns, so the sharp turns of plus ends are not likely to occur while the plus end is tracking along a stable microtubule. Instead, they probably represent a switch from a freely growing plus end to one that is following a track.

### Directed Growth and Neuronal Microtubule Polarity

Directed growth of microtubules at dendrite branch points allows dendrites to maintain uniform minus-end-out polarity despite continued microtubule remodeling. This mechanism is also probably necessary to establish uniform microtubule polarity in branched dendrites, but it probably cannot account for minus-end-out polarity on its own. We hypothesize that directed microtubule growth is used in concert with an unidentified mechanism to control microtubule polarity in dendrites. Transport of oriented microtubule pieces has been proposed to contribute to axon microtubule polarity [14], and a similar mechanism could play a role in dendrites. For example, a kinesin anchored to the cell cortex by its C terminus could shuttle minus-end-out microtubule seeds into dendrites. Alternatively, polarized microtubule nucleation sites could be localized within dendrites. Identifying directed growth as one mechanism that contributes to dendrite microtubule polarity should facilitate identification of the missing pieces of this puzzle.

### +TIPs, Kinesin-2, and Microtubule Polarity

Because kinesin-2, APC, EB1, and polarized microtubules are found in many cell types, directed growth of microtubules

along stable microtubule tracks could be very broadly used for maintaining microtubule polarity. This is a newly identified function for both the +TIP proteins and kinesin-2. APC has previously been localized to junctions between a microtubule tip and the side of another microtubule at the cortex of epithelial cells, and sliding of microtubule tips along the side of microtubules was also seen in this system, but there was no association with a particular direction of movement or overall microtubule polarity [39].

Kinesin-2 has previously been shown to be enriched in the tips of growing axons in cultured mammalian neurons [40], as has APC [41], and it is possible that they could mediate tracking of growing microtubules along existing microtubule tracks in the growth cone. In fact, this type of microtubule tracking behavior has been observed in axonal growth cones under low-actin conditions [42]. Thus, directed growth of microtubules could also be important during axon outgrowth. Indeed, the same principle of directed growth by a motor protein connected to a growing microtubule plus end could be involved in aligning microtubules in many circumstances.

## Experimental Procedures

### Drosophila Stocks

Many useful stocks were obtained from the Bloomington *Drosophila* Stock Center, the Vienna *Drosophila* RNAi Center (VDRC), National Institute of Genetics, Japan (NIG-Fly), Wes Grueber, and Dan Eberl. For details on these stocks, as well as transgenic *Drosophila* created for this study, see [Supplemental Information](#).

### Live Imaging of Larvae

All imaging of da neurons was performed in intact larvae mounted on a dried agarose pad under a coverslip. Neurons were imaged with a confocal microscope, either a Zeiss LSM510 or Olympus FV1000. For details on age, genotypes, and specific assays, see [Supplemental Information](#).

## Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and nine movies and can be found with this article online at [doi:10.1016/j.cub.2010.11.050](https://doi.org/10.1016/j.cub.2010.11.050).

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